DEVELOPMENT OF IMMUNE RESPONSES TO ASPERGILLUS AT AN EARLY AGE IN CHILDREN WITH CYSTIC FIBROSIS

Jane M. El-Dahr, M.D., Robert Fink, M.D., Robert Selden, M.D., L. Karla Arruda, M.D., Thomas A.E. Platts-Mills, M.D., Ph.D., and Peter W. Heymann, M.D.

From the Departments of Pediatrics and Internal Medicine, University of Virginia, Charlottesville, VA and the Department of Pediatrics, Tulane University School of Medicine, New Orleans, LA

Supported by National Institutes of Health Grants AI 24261 and AI 30840. Partial support was also provided by research funds from the Children's Medical Center, University of Virginia Health Sciences Center, Charlottesville, Virginia.

Reprint Requests: Jane M. El-Dahr, M.D.
Department of Pediatrics
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, LA 70112

Running Head: Immune Responses to Aspergillus in C.F.

20283882226

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

ABSTRACT

Although the ability of Aspergillus organisms to colonize the respiratory tract in patients with cystic fibrosis (CF) is well recognized, the contribution of Aspergillus to the disease process is poorly understood. Using sera from 147 CF patients (ages 5-43 years) we measured IgE antibody (ab) to *Aspergillus fumigatus* and 5 common inhalant allergens by radioallergosorbent test (RAST). Total IgE levels and IgG ab to radiolabelled *Asp f* I, an allergen purified from *Aspergillus fumigatus* and a potent inhibitor of protein synthesis, were also measured. Thirty (20%) of the patients had IgE ab to *A. fumigatus* and 22 (15%) of these patients had developed total IgE levels $\geq$ 400 IU/ml so that the diagnosis of Allergic Bronchopulmonary Aspergillosis (ABPA) might be considered. Five of the 22 patients developed these IgE responses by age 5 and fourteen by age 10. The proportion of patients with IgE ab to one or more of the other allergens tested was not significantly different from healthy controls (21% of 147 patients vs. 16% of 32 controls). A striking proportion (84%) of CF sera contained IgG ab to *Asp f* I compared to 6% of sera from control patients and 20% of sera from allergic children with asthma (n=25), only one of whom had IgE ab to *A. fumigatus*. Using additional sera from young CF patients, IgG anti-*Asp f* I ab was detected in 16 of 39 sera (41%) by age 5 increasing to 87 of 89 sera (98%) from patients older than age 10. The early development of immune responses to Aspergillus antigens, including the cytotoxic allergen *Asp f* I, raises the question as to whether Aspergillus, in addition to *Staphylococcus aureus* and *Pseudomonas aeruginosa*, contributes early to progressive lung deterioration in patients with CF.

2028388227

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

KEY WORDS:

Allergic Bronchopulmonary Aspergillosis (ABPA)

Cystic Fibrosis

Allergy

Aspergillus fumigatus

Fungal allergens

2028388228

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

INTRODUCTION

Previous studies of immediate hypersensitivity in patients with cystic fibrosis have consistently demonstrated an increased prevalence of sensitization to Aspergillus allergens (1-4). As judged by skin test responses, sensitization to allergens produced by Aspergillus species, in particular *A. fumigatus*, has been reported to be as high as 31 to 59% in the CF population (2-6). In those patients with IgE anti-Aspergillus ab, the diagnosis of ABPA, an intense inflammatory reaction to Aspergillus in the lungs, is considered when serum total IgE levels exceed 400 IU/ml (or 1000 ng/ml) in conjunction with deterioration in lung function (7). This diagnosis has been reported to affect as many as 10 to 12% of patients with CF (2,4,5) with up to 23% of CF patients meeting most but not all of the criteria (5,6). The increased risk for the development of IgE antibody to Aspergillus in CF patients is presumed to be related to the ability of these fungi to colonize the respiratory tract. Nelson and colleagues grew Aspergillus species from sputum of 57% of patients with CF; however, in other studies, culture yields have been variable and have been reported to be as low as 9 to 11% (2,4,8,9). Serologic responses to Aspergillus antigens generally indicate a higher rate of exposure. Serum precipitins have been reported in 30-51% of patients (2-5,8,9) and IgG anti-Aspergillus ab was demonstrated in 40-70% of CF sera by enzyme linked immunosorbent assay (ELISA) (10,11).

20283882229

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

As yet, it is not clear how early in life exposure and immune responses to Aspergillus antigens develop in CF or whether hypersensitivity to Aspergillus allergens, particularly in patients with high total IgE levels, contributes significantly to deterioration of lung function. In this study, we measured IgE ab to *A. fumigatus* and to 5 common inhalant allergens in sera from patients with CF by RAST and in a portion of these patients by skin prick tests. Results were compared with similar analyses in healthy controls and allergic children with asthma. Total serum IgE levels in all patients and sputum from patients who were skin tested were also obtained. Recently, a major allergen has been purified from *A. fumigatus*, *Asp f* I (12). This allergen shows extensive sequence homology (95%) with a cytotoxin (mitogillin) produced by *Aspergillus restrictus* and both proteins were shown to be potent inhibitors of protein synthesis. Using radiolabelled *Asp f* I, we measured antibody responses to this allergen in serum from all CF patients and controls.

METHODS

Population. Sera from 147 patients (ages 5-43 years, mean = 14.4) were analyzed. Stored sera were available from 97 of these patients (ages 5-26, mean = 13.0) and from an additional 25 younger patients (ages 12 months to 5 years) seen in the cystic fibrosis clinics at the Children's Hospital National Medical Center (Washington, D.C.) and the University of Virginia Children's Medical Center (Charlottesville, VA). Two or more samples, collected at different ages from 30 of these patients, were also available, primarily from children in

2028386230

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

the younger age group. Serum from each patient was obtained during a routine follow-up visit or hospitalization for pulmonary exacerbation and was stored at -20° C. In all patients, the diagnosis of CF had been confirmed by sweat chloride values of > 60 mEq/L by quantitative iontophoresis.

Fifty of the 147 patients (ages 5-43, mean = 18.2) were enrolled consecutively for skin testing together with serum analyses during routine visits to the CF clinic at the University of Virginia. Informed written consent was obtained from patients or their parents. In addition, 25 patients treated at the University of Virginia for asthma (ages 5-18 years, mean = 10.3) in the Pediatric Allergy Clinic and 32 children (ages 5-19 years, mean = 11.1) evaluated in the University of Virginia Pediatric Emergency Room for non-pulmonary diagnoses, and who required venipuncture for management, were enrolled as control patients. The protocol for this study was approved by the Investigational Review Board of the University of Virginia Health Sciences Center.

Immunoassays. Allergen specific IgE antibodies in sera were measured by quantitative RAST using cyanogen bromide activated filter discs coated with either a crude extract of *A. fumigatus* (Greer Laboratories, Lenoir, NC) or one of five other common inhalant allergens. Ten μg of protein from a 100% ammonium sulfate cut of the *A. fumigatus* extract, prepared as previously described (13), was coupled to each disc. The other allergens

2028388231

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

included *Dermatophagoides farinae* mite allergen, cat epithelium, short ragweed pollen, rye grass pollen, and mixed cockroach extracts (Hollister-Stier Laboratory, Spokane, WA). Approximately 0.1-0.5 μg of major allergen (e.g. *Der f* I, *Fel d* I, *Amb a* I, and *Lol p* I from mite, cat, ragweed, and grass extracts, respectively) were coupled to each disc. Allergen coated discs were incubated with dilutions (1/4 and 1/10) of patient's serum and IgE ab bound to allergen was detected using 2 ng of affinity purified ^{125}I-goat anti-human IgE (14). A reference control curve for each assay was established using *D. farinae* coated discs together with serial two-fold dilutions of serum pooled from eight mite allergic patients. This serum pool was allotted an activity of 1000 RAST units/ml by reference to a serum pool established at the National Institute of Biological Standards and Control in London (NIBSC 82/528), which contains 1,800 RAST units of IgE antibody to *D. farinae*. Each RAST unit is approximately equivalent to 0.1 ng of IgE. Dilutions of sera were carried out in 50% horse serum in PBS/TWEEN-20, pH 7.5. Two sera from non-allergic patients and two sera known to contain IgE ab specific for the allergen being tested were run in parallel with each assay. RAST scores of $\geq$ 40 RAST units/ml, or approximately 4 ng of allergen specific IgE ab/ml, were considered positive.

Serum IgG and IgE antibodies to the radiolabelled *Asp f* I were measured in an antigen binding RIA as previously described (12,15). Precipitins to Aspergillus antigens were identified using the standard gel double immunodiffusion template technique using

2028388232

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

unconcentrated serum (16). Each serum was tested against four sources of antigen: *Aspergillus fumigatus* Antigen #1 (20 mg protein/ml), Aspergillin (10 mg protein/ml), and a 1:20 w/v Aspergillus mix (*fumigatus, flavus, glaucus, nidulans,* and *niger*) each from Greer Laboratories, Lenoir, NC, and a 1:20 w/v Aspergillus mix from Hollister-Stier Laboratories, Spokane, WA.

Total serum IgE levels were measured using a mix of two different monoclonal anti-Fc-epsilon antibodies (CIA/E/7/12 and CIA/E/4.15, kindly provided by Dr. Andrew Saxon, UCLA) which were coupled to activated microtiter plates as previously described (14). Bound human IgE was detected using biotin labelled goat anti-IgE antibodies (Kirkegaard and Perry Labs, Gaithersburg, MD). Results were read from a standard curve using serum substandardized against an NIH standard serum containing 900 IU IgE/ml.

Skin testing. Prick skin testing was done on 50 CF patients and 25 allergic asthmatic patients enrolled consecutively in the University of Virginia Cystic Fibrosis and Pediatric Allergy Clinics, respectively. Extracts used were a 1:20 w/v Aspergillus mix, ragweed mix, 9 Southern grass mix, cat epithelium, and American cockroach from Greer Laboratories, Lenoir, NC and a 1:50 w/v *Dermatophagoides farinae* extract from Hollister-Stier Laboratories, Spokane, WA. Histamine (0.25 mg/ml) and saline prick tests were included as positive and negative controls, respectively. All patients had not taken an antihistamine

2028366233

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

for > 72 hours prior to testing. Results were assessed after 15 minutes. A wheal response with a diameter at least 3 mm greater than the negative control was read as positive.

Sputum. When obtained from CF patients, sputum was cultured on Sabouraud's Dextrose Agar with 100 μg/ml of gentamicin and 50 μg/ml of chloramphenicol added to inhibit growth of *Pseudomonas*. Plates were incubated at room temperature and positive fungal cultures were identified in the University of Virginia Hospital microbiology laboratory after 6 weeks. A complete blood count was also obtained from each patient to determine the absolute eosinophil count. Counts > 500 cells per cubic millimeter were considered elevated (7).

Pulmonary function testing. FEV_1 and FVC were performed on an S-model Vitalograph Spirometer (Kansas City, MO). Results were expressed as a percentage of predicted values for age, sex, race, and height based on the standards of Hsu et al (17). Four of the 50 patients enrolled at the University of Virginia were unable to perform spirometry because of acute pulmonary exacerbations. Spirometry values obtained from their most recent clinic visit were used.

2028388234

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Statistical analysis. Chi square analysis was used to compare the difference between percentages of CF patients and asthma patients with IgE ab to *A. fumigatus* and dust mite allergens. A two sample t-test on the log of geometric means was used to compare the difference between CF patients with and without IgE ab to *A. fumigatus* with respect to titers of IgG ab *Asp f* I.

RESULTS

Sensitization to Aspergillus and common inhalant allergens. As judged by skin prick tests and RAST analyses, allergen specific IgE ab was detected more commonly to Aspergillus allergens in the CF patients than to the other allergens tested (Table I).

Of the 49 patients who had IgE ab to one or more of these six allergens by RAST, 30 (61%) had IgE ab to Aspergillus, 17 of whom had IgE ab to Aspergillus alone.

If one excludes those sensitized to Aspergillus alone, then the prevalence of IgE ab to the inhalant allergens was similar in the CF population and control group (21% and 16%, respectively). By skin test analyses, positive prick tests to Aspergillus were observed in 68% of allergic CF who were skin tested patients compared to 4% of patients with allergic asthma (Fig. 1). By contrast, 80% of the asthmatic subjects had IgE antibody to *D. farinae* dust mite allergen compared to 23% of the skin test positive CF patients. The difference between CF patients and asthma patients with respect to the percentage of individuals with either Aspergillus or dust mite sensitivity was highly significant ($p < 0.001$).

2028388235

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Total IgE Levels and Suspected ABPA. In sera from the 30 patients who had anti-Aspergillus IgE antibody by RAST, 17 had total IgE levels $\geq$ 400 IU/ml (Table II). Eight of these patients had IgE ab to Aspergillus only. In the absence of IgE ab to Aspergillus, CF patients with inhalant allergy tended to have lower IgE levels. In addition to the 17 patients with total IgE level $\geq$ 400 IU/ml, five other patients with anti-Aspergillus IgE antibody had previous records of total IgE levels > 400 IU/ml which subsequently declined. Three of these five patients had not been treated with steroids. Thus 22 of 30 patients (73%) with IgE ab to Aspergillus, or 15% of the 147 patients ages 5 or older, had or did have immunologic responses characteristic of ABPA. Fourteen of these patients were $\leq$ age 10 and 5 patients were age 5 or younger. The diagnosis of ABPA, however, had been considered clinically by physicians in only 8 of the 22 patients.

Of the 50 patients screened with prick skin tests and total IgE levels, 15 had a positive test for Aspergillus (Table I) and 7 were suspected to have ABPA based on their total IgE levels. Two of the 50 patients had a total blood eosinophil count > 500, but only one of them had a positive Aspergillus skin test and an elevated total IgE (i.e. 690 IU/ml). Measurements of FEV_1 and FVC (and FEV_1/FVC ratios) in this same group did not differentiate the seven patients with suspected ABPA from the other patients.

2028382236

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

IgG antibodies to *A. fumigatus* and sputum cultures. The proportion of CF patients with serum IgG antibodies to the allergen *Asp f* I was 124/147 (84%). All 30 CF patients with serum IgE ab to Aspergillus by RAST had IgG anti-*Asp f* I ab. By contrast, only 5 of 25 (20%) of the allergic patients with asthma and 2 of 32 (6%) of children enrolled as healthy controls had detectable IgG ab to *Asp f* I (Fig 2). Titers of IgG ab to *Asp f* I were significantly higher in patients with IgE ab to *A. fumigatus* by RAST, including patients with total IgE ab $\geq$ 400 IU/ml, (geometric mean = 164.8 units/ml; 95% confidence limits 77.5 to 352.1 units/ml) than in CF patients who did not have IgE ab to Aspergillus (geometric mean = 47.8 units/ml; 95% confidence limits 34.9 to 67.0 units/ml), $p < 0.01$. In 21 sera from the 22 CF patients who had developed immune responses suggesting ABPA, 12 (57%) also showed IgE ab binding to *Asp f* I by antigen binding RIA.

Precipitins were detected in 15 (10%) of the 147 sera tested and twelve of these reacted with antigens present in the *A. fumigatus* Antigen #1 extract. All 15 sera also had IgG ab to *Asp f* I. Eight of the 15 had IgE ab to *A. fumigatus* by RAST and 6 of these had total IgE levels > 400 IU/ml. Forty of the 50 CF patients enrolled for skin testing were able to produce sputum. Aspergillus species were grown in 18 specimens (45%). Only 3 of these patients had total IgE ab $\geq$ 400 IU/ml. *A. fumigatus* was positively identified in 9 of the 18 cultures and came from patients who had high titers (> 100 units) of IgG ab to *Asp f* I.

20283882237

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Antibody Responses to Aspergillus in young patients. Prior to age 3, the prevalence of IgG ab to the allergen *Asp f* I was low (13%) (Fig. 3). After age 3, a marked increase in the percentage of patients with IgG anti-*Asp f* I ab developed such that after age 10, 98% of patients had detectable antibody responses. A progressive increase with age in titers of IgE ab to Aspergillus by RAST was also evident in sera from 58 CF patients $\leq$ age 10 (Fig. 4A). Fifteen of these patients had developed total IgE levels $\geq$ 400 IU/ml, 14 of whom had IgE ab to Aspergillus (Fig. 4B). In Figure 4A, titers of IgE ab to Aspergillus in serial serum samples (connected circles) available from 9 of these patients are also shown. Six of these patients developed titers > 200 RAST units/ml as well as total IgE levels $\geq$ 400 IU/ml. Five patients developed these levels by age 5 (Fig. 4B).

DISCUSSION

Allergens produced by *A. fumigatus*, and other Aspergillus species, have been shown to be potent stimuli for IgE ab production in patients with CF. Our results demonstrate further that these vigorous IgE responses to Aspergillus in CF can develop at an early age as do immune responses to the *A. fumigatus* allergen and cytotoxic protein, *Asp f* I. As judged by prick skin tests and measurements of serum IgE ab, sensitization to Aspergillus allergens in our patient population was markedly increased compared to other common allergens studied (mite, cat, cockroach, rye grass, and ragweed). Similar to previous reports, we were not able to demonstrate that the percentage of CF patients sensitized to these other allergens

2028368238

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

was significantly increased compared to controls (5,18), whereas the proportion of CF patients sensitized to *A. fumigatus* allergen was significantly increased compared to both asthma and control patients. Twenty-two (15%) of patients older than age 5 in our study had developed IgE ab to Aspergillus in association with high total IgE ab ($\geq$ 400 IU/ml) and IgG ab suggesting the possible diagnosis of ABPA in these individuals. These immune responses were detected in 14 of the 22 patients by the age of 10 and in five patients by the age of 5.

In patients with asthma, the diagnosis of ABPA is considered when IgE ab to Aspergillus and high total IgE ab occur together with other clinical and immunological manifestations (e.g. wheezing, recurrent pulmonary infiltrates, central bronchiectasis, peripheral eosinophilia, and serum precipitins) (7). In patients with cystic fibrosis, however, the diagnosis is more challenging. First, recurrent pneumonic infiltrates and bronchiectasis occur, making it difficult to judge pulmonary manifestations of ABPA using standard x-ray techniques (4). Second, airway hyperreactivity requiring treatment with bronchodilators and sometimes steroids is also common in CF and often is associated with infectious processes (19). Third, over time some CF patients with serologic evidence for ABPA can show variable immune responses to Aspergillus (e.g. loss of precipitins and the fluctuations of serum IgE and IgG ab to Aspergillus) as well as a marked decline in their total IgE levels over time without having been treated with steroids (20). In keeping with

2028388239

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

these diagnostic problems, three of the 22 patients in our study who had developed the antibody characteristics of ABPA had shown a spontaneous decline in their total IgE levels without steroid treatment, and only 8 had been clinically suspected to have the disorder. In addition, peripheral blood eosinophilia was not a marked feature in our patients similar to a previous report of CF patients with suspected ABPA (4).

Although it has been difficult to culture Aspergillus fungi from sputum obtained from CF patients, Aspergillus is thought to colonize and persist in the respiratory tract (3,5). Thus, exposure to Aspergillus in the respiratory tract is the most likely explanation for the high incidence of IgE and IgG ab to Aspergillus allergen. Consistent with a high incidence of exposure, 89% of patients in our study had serum IgG ab to *Asp f* I allergen which was very high compared to previous reports of serum IgG ab responses to Aspergillus in CF patients measured by precipitins or ELISA (2-5,7-10). Furthermore, the detection of IgG ab to *Asp f* I in sera from 39% of our patients by age 5 strongly suggests that exposure to Aspergillus fungi begins at an early age. It is possible that genetic factors could predispose CF patients to respond to Aspergillus. However, the CF gene is localized on chromosome 7 (21,22) whereas allergen specific IgE ab responses to ragweed and rye grass allergens have been reported to be associated with HLA-DR loci located on chromosome 6 (23). A gene locus associated with increased IgE ab responses to inhaled allergens has been reported on chromosome 11 (24). Thus, a linkage disequilibrium between CF and allergen specific immune responses appears to be unlikely based on current information.

2028388240

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

The predilection of *Pseudomonas aeruginosa* for the lungs of patients with cystic fibrosis is well recognized (25). The same might be proposed for Aspergillus fungi considering the high rate of immune responses to *Asp f* I and growth of Aspergillus in 45% of sputum cultures in our study. Recently, *Asp f* I has been shown to be an 18 kd secreted protein which is not detected in significant amounts in Aspergillus spores or mycelial components (15). It is also a potent inhibitor of protein synthesis (26). Other toxic agents in *A. fumigatus* extracts have been reported including metabolites with anti-phagocytic properties (27-29) and a proteinase able to induce epithelial cell detachment from basement membrane (30). Although local factors in the lung may create a suitable growth environment for Aspergillus, the secretion of toxins including *Asp f* I may further enhance the ability of Aspergillus to colonize and persist in the respiratory tract. Although the potential pathologic consequences of Aspergillus, thus far, have focused on CF patients who develop vigorous IgE antibody responses, our data raise the question as to whether exposure to Aspergillus may contribute to chronic and gradual pulmonary deterioration in CF.

In practice, the diagnosis of ABPA is often suspected in CF patients who have a positive skin test and/or serum IgE ab to Aspergillus together with a high total IgE level, particularly when a lung infiltrate is present and the patient's respiratory status fails to improve with appropriate antibiotic and supportive therapy. Oral steroids still represent the basic approach to management in these patients, even though long-term courses of steroids

20283886241

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

have well known complications. As yet there is little information available about the efficacy of oral anti-fungal agents, such as ketoconazole and itraconazole, in treating CF patients with suspected ABPA. In the future the use of antifungals which do not cause unacceptable side effects may help clarify the pathophysiologic significance of Aspergillus in these patients. The use of specific anti-fungal treatment would also permit studies to assess whether Aspergillus, because of its ability to secrete *Asp f I* and other cytotoxic metabolites, may also have pathogenic consequences in the lungs of CF patients who were shown to develop immune responses to Aspergillus at an early age.

2028388242

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

ACKNOWLEDGEMENT

We are very grateful to Eliza T. Platts-Mills for technical assistance, and to Steven J. Savitt and Marcella A. Wise for help in preparing this manuscript.

2028368243

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

REFERENCES

1. Warren CPW, Tai E, Batten JC, Hutchcroft BJ, Pepys J. Cystic fibrosis-immunological reactions to A. fumigatus and common allergens. Clin Allergy 1975; 1:1-12.

2. Nelson LA, Callerame ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. Am Rev Respir Dis 1979; 120:863-73.

3. Zeaske R, Bruns T, Fink JN, Greenberger PA, Colby H, Liotta JL, Roberts M. Immune responses to Aspergillus in cystic fibrosis. J Allergy Clin Immunol 1988; 82:73-7.

4. Laufer P, Fink JN, Bruns T, Unger GFR, Kalbfleisch JH, Greenberger PA, Patterson R. Allergic bronchopulmonary Aspergillosis in cystic fibrosis. J Allergy Clin Immunol 1984; 73:44-8.

5. Feanny S, Forsyth S, Corey M, Levison H, Zimmerman B. Allergic bronchopulmonary Aspergillosis in cystic fibrosis: a secretory immune response to a colonizing organism. Ann Allergy 1988; 60:64-8.

6. Valletta EA, Braggion C, Mastella G. Sensitization to Aspergillus and Allergic Bronchopulmonary Aspergillosis in a cystic fibrosis population. Ped Asthma, Allergy & Immunol 1993; 7:43-9.

2028388244

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

93-0493

7. Greenberger PA, Patterson R. Diagnosis and management of allergic bronchopulmonary Aspergillosis. Ann Allergy 1986; 56:444-48.

8. Mearns M, Longbotton J, Batten J. Precipitating antibodies to *Aspergillus fumigatus* in cystic fibrosis. Lancet 1967; 1:538-9.

9. Schwartz RH, Johnstone DE, Holschaw DS, Dooley RR. Serum precipitins to *Aspergillus fumigatus* in cystic fibrosis. Am J Dis Child 1970; 120:432-3.

10. Edwards JH, Alfaham V, Fifields R, Philpot C, Clement MJ, Goodchild MC. Sequential serologic responses to Aspergillus fumigatus in patients with cystic fibrosis. Use of antigen 'stretching' to delineate IgG and IgE activity. Clin Exp Immunol 1990; 81:101-8.

11. Forsyth KD, Hohmann AW, Martin AJ, Bradley J. IgG antibodies to *Aspergillus fumigatus* in cystic fibrosis: a laboratory correlate of disease activity. Arch Dis Child 1988; 63:953-57.

12. Arruda LK, Platts-Mills TAE, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the Mitogillin family of cytotoxins. J Exp Med 1990; 172:1529-32.

2028388245

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

13. Harvey C, Longbottom JL: Characterization of a major antigenic component of *Aspergillus fumigatus*. Clin Exp Immunol 1986; 65:206-14.

14. Pollart SM, Chapman MD, Fioico GP, Rose G, Platts-Mills TAE: Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83:875-82.

15. Arruda LK, Platts-Mills TAE, Longbottom JL, El-Dahr JM, Chapman MD. *Aspergillus fumigatus*: identification of 16, 18, and 45 KD antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. J Allergy Clin Immunol 1992; 89:1166-76.

16. Kurup VP, Fink JN. Evaluation of methods to detect antibodies against *Aspergillus fumigatus*. Am J Clin Pathol 1978; 69:414-7.

17. Hsu KHK, Jenkins DE, Bartholomew PH, Bourhofer E, Thompson V, Tanakawa N, Hsieh GSJ: Ventilatory functions of normal children and young adults--Mexican-American, white and black. I. Spirometry. J Pediatr 1979; 95:14-23.

18. Silverman M, Hobbs FDR, Gordon IRS, Carswell F. Cystic fibrosis, atopy, and airways lability. Arch Dis Child 1978; 53:873-7.

2028368246

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

19. Stern RC. The primary care physician and the patient with cystic fibrosis. J Pediatr 1989; 114:33-6.

20. Hutcheson PS, Rejent AJ, Slavin RG. Variability in parameters of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. J Allergy Clin Immunol 1991; 88:390-4.

21. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, Zsiga M, Buchwald M, Riordan JR, Tsui LC, Collins FS. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989; 245:1059-65.

22. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou J, Drumm ML, Iannuzzi MC, Collins FS, Tsui L. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245:1066-73.

23. Marsh DG, Hsu SH, Hussain R, Meyers DA, Friedhoff MS, Bias WB. Genetics of human immune response to allergens. J Allergy Clin Immunol 1980; 65:322-32.

24. Cookson WOCM, Faux JA, Sharp PA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11 q. Lancet 1989; 1:1292-5.

2028388247

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

25. Davis PB. Cystic fibrosis from bench to bedside. N Engl J Med 1991; 325:575-7.

26. Arruda LK, Mann BJ, Chapman MD. Selective expression of a major allergen and cytotoxin, *Asp f* I, in *Aspergillus fumigatus*: implications for the immunopathogenesis of Aspergillus related diseases. J Immunol 1992; 149, 3354-59.

27. Kurup VP, Resnick A, Scribner GH, Gunasekaran M, Rink JN. Enzyme profile and immunochemical characterization of *Aspergillus fumigatus* antigens. J Allergy Clin Immunol 1986; 78:1166-73.

28. Mullbacher A, Waring P, Eichner RD. Identification of an agent in cultures of *Aspergillus fumigatus* displaying anti-phagocytic and immunomodulating activity in vitro. J Gen Microbiol 1985; 131:1251-58.

29. Yokota K, Shimada H, Kamaguchi A, Sakaguchi O. Studies on the toxins of *Aspergillus fumigatus*. Microbiol Immunol 1977; 21:1-16.

30. Robinson BWS, Venaille TJ, Mendis AHW, McAller R. Allergens as proteases: an Aspergillus fumigatus proteinase directly induces human epithelial cell detachment. J. Allergy Clin Immunol 1990; 86:726-31.

2028366248

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Table I. Allergen Specific IgE Antibody Responses*

	CYSTIC FIBROSIS		CONTROL PATIENTS
A. Allergens Tested	Skin Test ($n=50$)†	RAST($n=147$)‡	RAST ($n=32$)§
Aspergillus species‖	15 (30%)	30 (20%)	1 (3%)
mite (*D. farinae*)	5 (10%)	14 (10%)	4 (13%)
cat	4 (8%)	20 (14%)	1 (3%)
ragweed	8 (16%)	7 (5%)	1 (3%)
rye grass	5 (10%)	10 (7%)	3 (9%)
cockroach	0	0	0
B. Patients with IgE ab to:			
At least one allergen tested	22 (44%)	49 (33%)	5 (16%)
Inhalant allergens	12 (24%)	31 (21%)	5 (16%)
and *Aspergillus*¶			
Aspergillus only	10 (20)%	17 (12%)	0
Inhalant allergens only**	7 (14%)	19 (13%)	4 (12.5%)

2028388249

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Footnotes for Table I

* The number of positive tests, followed by the percentage of tests positive in parentheses, are shown for each allergen tested.

† Prick skin tests were carried out on 50 patients with cystic fibrosis (≥ age 5) enrolled consecutively at the University of Virginia's Children's Medical Center. A wheal diameter at least 3 mm > than the negative control was regarded as positive.

‡ Sera for RAST analyses were obtained from 97 patients seen at the Children's Hospital National Medical Center, Washington, D.C., and 50 patients seen at the University of Virginia Children's Medical Center. All patients were ≥ age 5. Values ≥ 40 RAST mites/ml were regarded positive.

§ Sera for control patients were obtained from children (≥ age 5) seen in the University of Virginia Pediatric Emergency Room for a chief complaint which did not involve the respiratory tract.

‖ An extract of mixed *Aspergillus* species (*fumigatus, flavus, glaucus, nidulans,* and *niger*) was used for skin testing. An extract of *A. fumigatus* was used for the RAST analyses.

¶ Patients in this group had IgE ab to one or more of the five inhalant allergens tested and some of these patients also had IgE ab to *Aspergillus.*

** Patients in this group had IgE ab to one or more of the five allergens tested, but did not have IgE ab to *Aspergillus.*

20283882250

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Table II. Correlation between Serum IgE Antibody to Inhalant Allergens and Total IgE Levels in Cystic Fibrosis.

	Total IgE (IU/ml)*		
	>400	100-400	<100
Patients with IgE ab to:	(n = 19)	(n = 24)	(n = 104)
At least one allergen tested	18 (95%)	13 (54%)	18 (17%)
Inhalant allergens and *A. fumigatus*	17 (89%)	7 (29%)	6(6%)
A. fumigatus only	8 (42%)	5 (21%)	4 (4%)
Inhalant allergens only	1 (5%)	6 (25%)	12 (12%)

2028388251

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Footnotes for Table II

* Total serum IgE levels were measured using a two-site monoclonal antibody RIA specific for F_c-ϵ (12).

2028386252

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Legends for Figures:

Figure 1: Distribution of skin test results in allergic patients with cystic fibrosis (solid bars) and asthma (hatched bars). Prick skin tests were positive to one or more allergens tested in 22 of 50 CF patients ($\geq$ age 5) enrolled consecutively at the University of Virginia Health Sciences Center. Results in these 22 patients were compared to skin responses in 25 allergic asthmatic children ($\geq$ age 5) tested in the Pediatric Allergy Clinic at the University of Virginia. Allergen extracts used were Aspergillus, dust mite (*D. farinae*), ragweed, rye grass, cat, and cockroach.

Figure 2: Measurements of IgG antibody to *Asp f* I in sera from 147 patients with CF, 25 patients with asthma and 32 controls. IgG antibodies were measured by radio-immunoprecipitation. Sera with IgE antibodies to *A. fumigatus* by RAST are indicated by solid symbols (i.e. *A f* IgE POS). Solid squares indicate patients who also developed a total IgE level $\geq$ 400 IU/ml. Open circles represent patients who were RAST negative to *A. fumigatus* (i.e. *A f* IgE NEG). Sera with $\leq$ 5 units/ml of IgG ab to *Asp f* I (i.e. less than 2 standard deviations above the mean IgG binding to *Asp f I* in control sera) were regarded as negative. Geometric means (indicated by hatched lines) for CF patients who were *A f* IgE POS and *A f* IgE NEG were 164.8 units/ml and 47.8 units/ml, respectively, and were significantly different, $p < 0.01$.

2028388253

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

Figure 3: Prevalence, with respect to age, of serum IgG antibodies to *Asp f* I in patients with cystic fibrosis. Sera from 147 patients $\geq$ age 5 together with sera from 25 patients < age 5 were analyzed. Two or more samples, obtained at different ages, were available from 30 of the patients. The age ranges indicated along the abscissa span periods of 2 years (e.g. 1-2 is the same as 12 months through 2 years, 11 months).

Figure 4a: Development of IgE antibodies to *A. fumigatus* in patients with cystic fibrosis. Results are shown for all sera tested from children 10 years old or younger. Results of serial serum samples available from 9 patients are shown by connected circles. Closed circles represent RAST measurements $\geq$ 40 units/ml (i.e. above hatched line) and were regarded positive. Numbers along the abscissa followed by an X represent sera tested at each age with measurements < 20 RAST units/ml.

Figure 4b: Development of total IgE levels in patients with cystic fibrosis. Results are shown for all sera tested in Figure 4a with serial samples represented by connected symbols. Sera which were positive by RAST to *A. fumigatus* are shown as closed circles. Those which were RAST negative to *A. fumigatus*, but positive to one or more of the five other allergens tested, are indicated by open squares and those negative to all six allergens are indicated by open triangles. The (*) denotes a decline in total IgE observed following treatment of one of the patients with steroids. Numbers along the abscissa followed by a triangle represent sera tested at each age with < 10 IU/ml of total IgE.

20263682254

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

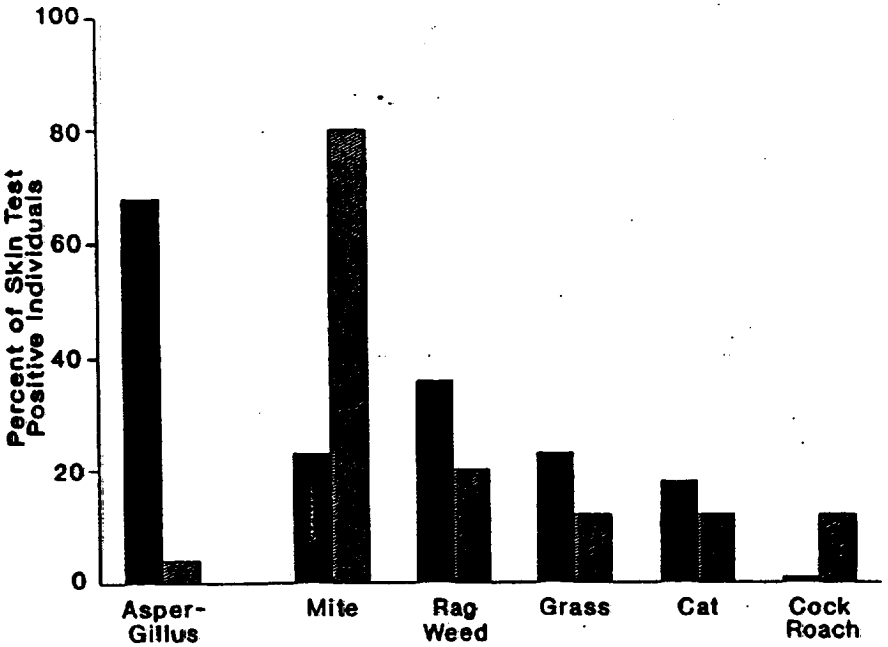


Fig. 1

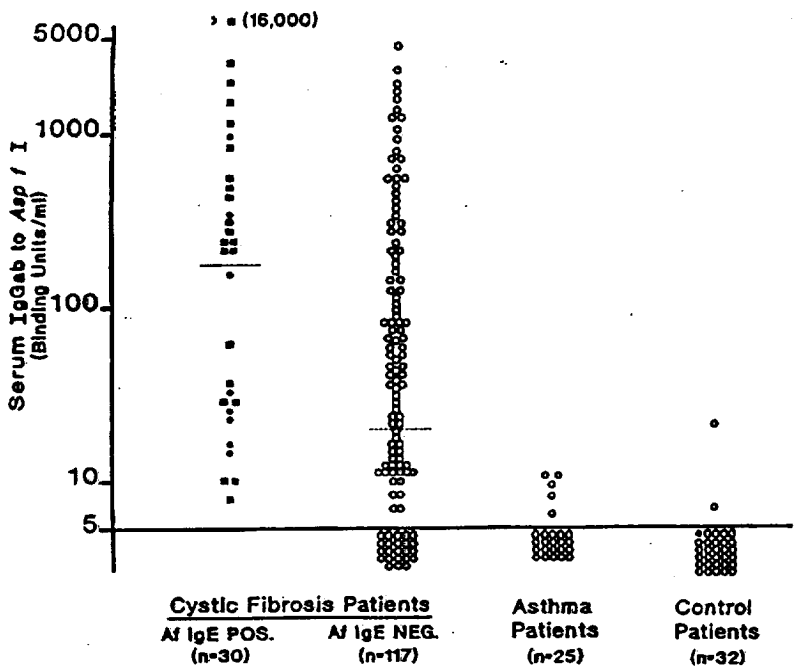


Fig. 2

20230808255

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

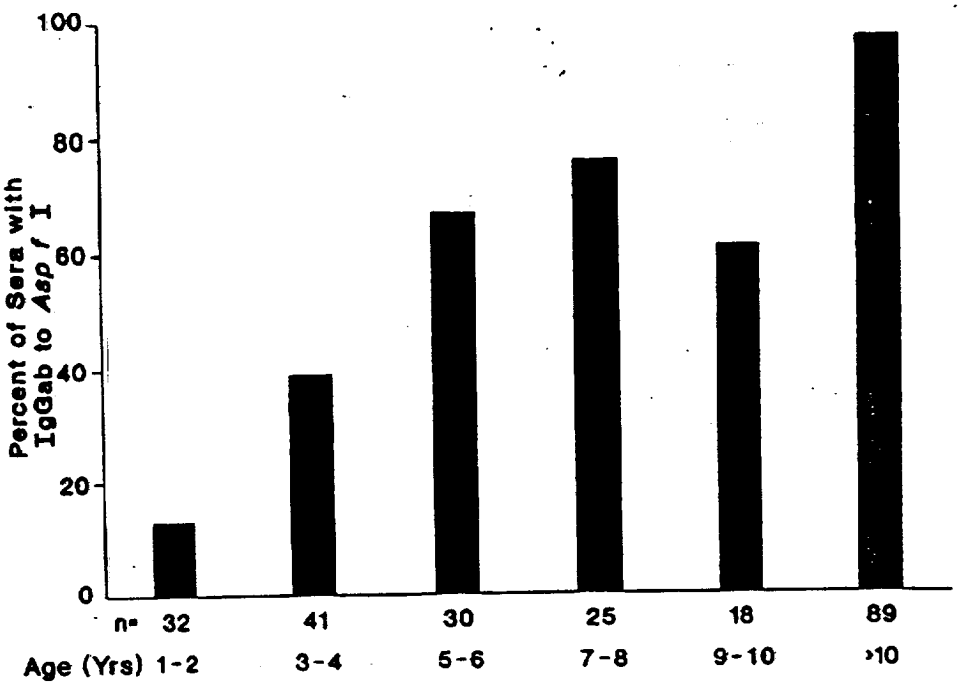


Fig. 3

20283882S6

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000

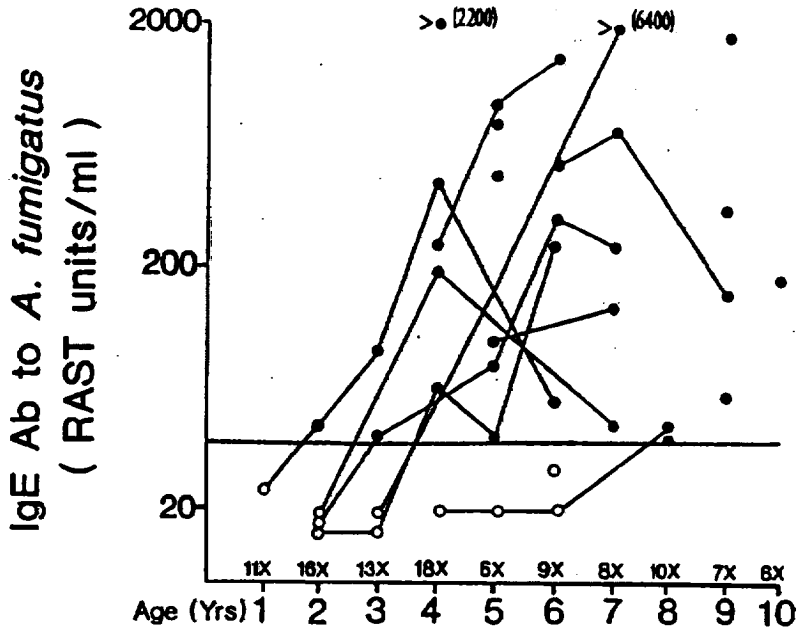
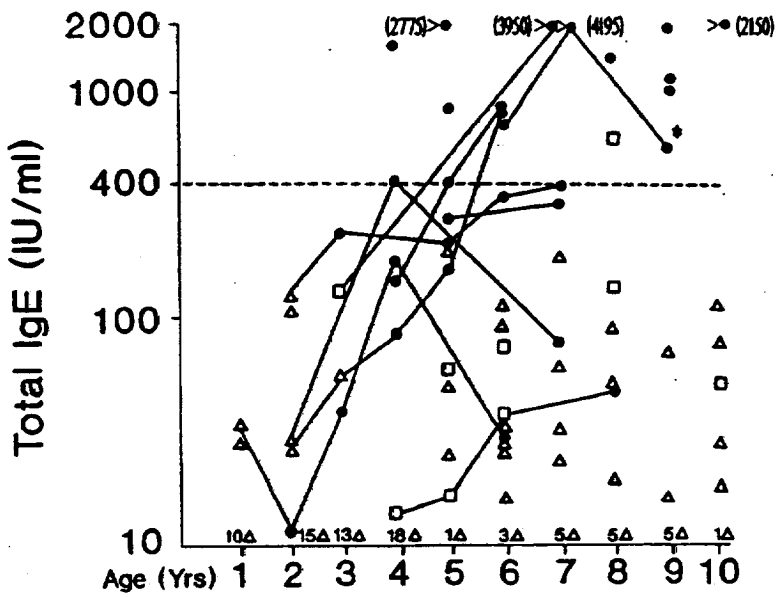


Fig. 4A



20283882257

Fig. 4B

Source: https://www.industrydocuments.ucsf.edu/docs/pknm0000